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(54) **LABELED MOLECULAR IMAGING AGENTS
AND METHODS OF USE**

FOREIGN PATENT DOCUMENTS

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WO 2006096207 A2 9/2006
WO 2011057986 A1 5/2011
WO 2011061154 A1 5/2011
WO WO 2012150220 A1 * 11/2012

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OTHER PUBLICATIONS

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patent is extended or adjusted under 35
U.S.C. 154(b) by 232 days.

Wang, J-Q et al. Bioorg. Med. Chem. 2005, 13, 5779-5786.*
Wester et al. Nucl. Med. Biol. 1999, 26, 259-265.*
Sirion et al. Tetrahedron Lett. 2007, 48, 3953-3957.*
Banjac et al., "The Cystine/Cysteine Cycle: A Redox Cycle Regu-
lating Susceptibility Versus Resistance to Cell Death", Oncogene,
vol. 27, pp. 1618-1628, 2008.
Bassi et al., "Identification and Characterisation of Human xCT
That Co-Expresses, With 4F2 Heavy Chain, the Amino Acid Trans-
port Activity System xc", Pflügers Arch—Cur J Physiol, vol. 442,
pp. 286-296, 2001.
Dai et al., "Chemoinformatics Analysis Identifies Cytotoxic Com-
pounds Susceptible to Chemoresistance Mediated by Glutathione
and Cystine/Glutamate Transport System xc", J. Med. Chem., vol.
50, pp. 1896-1906, 2007.
Gout et al., "Sulfasalazine, a Potent Suppressor of Lymphoma
Growth by Inhibition of the xc Cystine Transporter: A New Action
for an Old Drug", Leukemia, vol. 15, pp. 1633-1640, 2001.
Huang et al., "Cystine-Glutamate Transporter SLC7A11 in Cancer
Chemoresensitivity and Chemoresistance", Cancer, Res, vol. 65, No.
16, pp. 7446-7454, Aug. 15, 2005.
Kim et al., "Human Cystine/Glutamate Transporter: cDNA Cloning
and Upregulation by Oxidative Stress in Glioma Cells", Biochimica
et Biophysica Acta, vol. 1512, pp. 335-344, 2001.
Lackman et al., "Innate Immune Recognition Triggers Secretion of
Lysosomal Enzymes by Macrophages", Traffic, vol. 8, pp. 1179-
1189, 2007.
Lo et al., "The xc Cystine/Glutamate Antiporter: A Mediator of
Pancreatic Cancer Growth With a Role in Drug Resistance", British
Journal of Cancer, vol. 99, pp. 464-472, 2008.
Mawatari et al., "Reactive Oxygen Species Involved in the Gluta-
mate Toxicity of C6 Glioma Cells Via XC Antiporter System",
Neuroscience, vol. 73, No. 1, pp. 201-208, 1996.
Patel et al., "Differentiation of Substrate and Non-Substrate Inhi-
bitors of Transport System xc: An Obligate Exchanger of L-Glutamate
and L-Cystine", Neuropharmacology, vol. 46, pp. 273-284, 2004.
Plathow et al., "Tumor Cell Metabolism Imaging", The Journal of
Nuclear Medicine, vol. 49, No. 6, pp. 43S-63S, Jun. 2008.
Sato et al., "Induction of Cystine Transport Activity in Mouse
Peritoneal Macrophages by Bacterial Lipopolysaccharide",
Biochem. Journal, vol. 310, pp. 547-551, 1995.

(Continued)

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(56) **References Cited**

U.S. PATENT DOCUMENTS

5,171,563 A 12/1992 Abrams et al.
6,306,911 B1 10/2001 Wachter et al.
6,953,567 B2 10/2005 Griffiths
7,196,063 B1 3/2007 Shirvan et al.
7,211,240 B2 5/2007 Arbogast et al.
7,279,170 B2 10/2007 Content et al.
7,902,332 B2 3/2011 De Jesus et al.
7,906,103 B2 3/2011 Graupner
8,486,370 B2 7/2013 Carpenter et al.
8,784,774 B2 7/2014 Webster et al.
2002/0115688 A1* 8/2002 Beart et al. 514/312
2005/0276795 A1 12/2005 Batarseh
2007/0014719 A1 1/2007 Reading et al.
2009/0022664 A1 1/2009 Srinivasan et al.
2009/0226913 A1 9/2009 Pope et al.
2010/0021379 A1 1/2010 Lam et al.
2010/0272641 A1 10/2010 Webster et al.
2010/0324008 A1 12/2010 Low et al.
2011/0112033 A1 5/2011 Gluckman et al.
2011/0123976 A1 5/2011 Raftery et al.
2011/0165076 A1 7/2011 Dinkelborg et al.
2012/0093724 A1 4/2012 Denmeade et al.

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(57) **ABSTRACT**

Imaging agents are described that comprise labeled sub-
strates capable of being introduced into cells via the cystine/
glutamate antiporter. The substrates may be used for imag-
ing or detecting oxidative stress in cells by introducing the
labeled agents into cells via the cystine/glutamate antiporter
and subsequent detection.

7 Claims, No Drawings

(56)

References Cited**OTHER PUBLICATIONS**

Sato et al., "Induction of Cystine Transport Via System xc and Maintenance of Intracellular Glutathione Levels in Pancreatic Acinar and Islet Cell Lines", *Biochimica et Biophysica Acta*, vol. 1414, pp. 85-94, 1998.

Sato et al., "Cloning and Expression of a Plasma Membrane Cystine/Glutamate Exchange Transporter Composed of Two Distinct Proteins", *The Journal of Biological Chemistry*, vol. 274, No. 17, pp. 11455-11458, 1999.

Varagnolo et al., "F-Labeled Radiopharmaceuticals for PET in Oncology, Excluding FDG", *Nuclear Medicine & Biology*, vol. 27, pp. 103-112, 2000.

Wu et al., "Glutathione Metabolism and Its Implications for Health", downloaded from jn.nutrition.org, pp. 489-492, 2003.

Taguchi et al., "Induction of Cystine/Glutamate Transporter in Bacterial Lipopolysaccharide Induced Endotoxemia in Mice", *Journal of Inflammation*, vol. 4, No. 20, pp. 1-7, 2007.

Mease et al., "N-[N-[(S)-1,3-Dicarboxypropyl]Carbamoyl]-4-[18F]Fluorobenzyl-L-Cysteine, [18F]DCFBC: A New Imaging Probe for Prostate Cancer", *Clin Cancer Res*, vol. 14, No. 10, pp. 3036-3043, May 15, 2008.

De Bruin et al., "1-[3-(2-[18F]Fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione: Design, Synthesis, and Radiosynthesis of a New [18F]Fluoropyridine-Based Maleimide Reagent for the Labeling of Peptides and Proteins", *Biconjugate Chem.*, vol. 16, pp. 406-420, 2005.

Berndt et al., Labeling of Low-Density Lipoproteins Using the 18F-Labeled Thiol-Reactive Reagent N-[6-(4-[18F]Fluorobenzylidene)Aminoxyhexyl]Maleimide, *Nuclear Medicine and Biology*, vol. 34, pp. 5-15, 2007.

Poethko et al., "Chemoselective Pre-Conjugate Radiohalogenation of Unprotected Mono- and Multimeric Peptides Via Oxime Formation", *Radiochim. Acta*, vol. 92, pp. 317-327, 2004.

Koglin et al., "Specific PET Imaging of xc Transporter Activity Using a 18F-Labeled Glutamate Derivative Reveals a Dominant Pathway in Tumor Metabolism", *Clinical Cancer Research*, Abstract.

Mitra et al., "Studies of the 18F L-Glutamate Derivative BAY 94-9392 in Cancer Patients; A Novel Radiopharmaceutical for PET Imaging", *J. Nucl. Med.*, vol. 52, (Supplement 1): 1900, 2011, Abstract.

Ploessl et al., "Comparison of Cell Uptake of Fluorine-18 Labeled (2S,4R)-4-Fluoro-Glutamine (FGIn) and (2S,4R)-4-Fluoro-Glutamic Acid (FGlu)", *Nucl. Med.*, vol. 52, (Supplement 1): 1569, 2011, Abstract.

Baek et al., "First Experience With Bay 94-9392, A Novel F-18 L-Glutamate Derivative, for PET/CT Imaging in Patients With Non-Small Cell Lung and Breast Cancer", *J. Nucl. Med.*, vol. 52, (Supplement 1): 195, 2011, Abstract.

Koglin et al., "Bay 94-9392—A Novel F-18 L-Glutamate Derivative for Tumor-Specific PET Imaging", *J. Nucl. Med.*, vol. 52, (Supplement 1): 412, 2011, Abstract.

Smolarz et al., "Bay 94-9392: A Novel F-18 Labeled Tumor Specific Probe for PET/CT Imaging—Dosimetry", *J. Nucl. Med.*, vol. 52, (Supplement 1): 1465, 2011, Abstract.

Search Report and Written Opinion from corresponding PCT Application No. PCT/US2012/053861 dated Nov. 19, 2012.

Hijarrubia et al., "Domain structure characterization of the multi-functional [alpha]-amino adipate reductase from *Penicillium chrysogenum* by limited proteolysis: Activation of [alpha]-amino adipate does not require the peptidyl carrier protein box or the reduction domain", *Journal of Biological Chemistry*, vol. 278, No. 10, pp. 8250-8256, Mar. 7, 2003.

O'Sullivan et al., "Incorporation of 3H from delta-(L-alpha-amino (4,5-3)adipyl)-L-cysteinyl-D-(4,4-3H)valine into isopenicillin N", *Biochem J.*, vol. 182(2), pp. 421-426, 1979.

Ploessl et al., "Comparative Evaluation of 18F-Labeled Glutamic Acid and Glutamine as Tumor Metabolic Imaging Agents", *The Journal of Nuclear Medicine*, vol. No. 53, Issue No. 10, pp. 1616-1624, Oct. 2012.

PCT Search Report and Written Opinion issued in connection with corresponding Application No. PCT/US2015/010429 on Apr. 10, 2015.

* cited by examiner

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LABELED MOLECULAR IMAGING AGENTS AND METHODS OF USE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with Government support under contract number 5R01EB014250-02 awarded by the National Institute of Health (NIH). The Government has certain rights in the invention.

BACKGROUND

The invention relates generally to labeled molecular imaging agents and more particularly to imaging agents that are taken up by the cells via the cystine/glutamate transporter.

The concept of molecular imaging promises specific contrast enhancement of molecular signatures of pathology and requires targetable biomarkers that are specifically regulated in certain pathological indications. While such a specific molecular contrast agent could have great utility for imaging and diagnosing disease; validation of a truly specific biomarker has proven to be very difficult. Even if an agent to such a specific biomarker is created, the market for such an agent will be limited to the prevalence of this indication. Therefore there is great interest in developing molecular contrast agents that can be utilized to image a variety of pathological indications. Most imaging agents target specific tissue or cell types, or specific therapies, or they degrade rapidly over time. One example of an agent that is directed at broader applications is ^{18}F -fluorodeoxyglucose (FDG) that makes use of the glucose transporter. ^{18}F -FDG is preferentially taken up by cells that have an increased requirement for glucose, and then is trapped inside the cell. FDG can be used clinically for the diagnosis, staging and monitoring of many cancers as well as monitoring metabolism in the heart and brain. ^{18}F -FDG is not a substrate for sodium-dependent glucose transporters found in the kidney tubules, which prevents its renal resorption and enhances clearance.

In vivo oxidative stress is recognized as an indicator of cellular stress. Efforts to image this stress have involved imaging animals using electron paramagnetic resonance (EPR). EPR is a technique for detecting unpaired electrons as would occur with the creation of free radicals in oxidative stress. Essentially an agent is used which is considered to be an EPR probe which is sensitive to organ antioxidant activity as a measure of oxidative stress.

Others have looked at using a ^{13}C -glycine chemical shift MRI to detect glycine uptake and conversion to glutathione in an animal model of chemotherapy treatment of tumors in vivo. Still others, having developed imaging agents to detect apoptotic cells in vivo for monitoring chemotherapy treatment (e.g. labeled Annexin V which is a rather large protein, Aposense by Neurosurvival Technologies which is a family of small molecules which is reported to enter specifically into only apoptotic cells.

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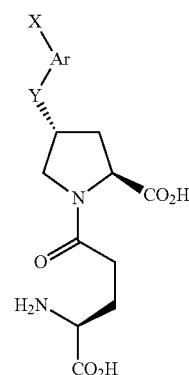
Also reported are imaging agents that take advantage of the cellular amino acid transporter (cystine/glutamate antiporter, x_c^-), which is activated under conditions of cellular oxidative stress. This is described in U.S. patent application Ser. No. 12/430,573 filed on Apr. 27, 2009 and U.S. patent application Ser. No. 13/234,210 filed on Sep. 16, 2011 which are incorporated herein by reference.

It advantageous to further exploit the cystine/glutamate antiporter transport mechanism using other substrates which take advantage of the transport mechanism with other properties. For example, structures that can be readily modified using standard radiofluorination techniques are preferred as it would allow a precursor to be supplied and prepared at a point of care. Furthermore structures, which can be prepared as a single enantiomer as compared to a racemic mixture may be preferable for use as a pharmaceutical agent.

BRIEF DESCRIPTION

The imaging agents and methods of the invention take advantage of the cellular amino acid transporter (cystine/glutamate antiporter, x_c^-) which is activated under conditions of cellular oxidative stress. Additionally, the upregulation of the cystine/glutamate transporter is also associated with chemotherapy resistance in some tumors. Therefore, non-invasive imaging of tumors with high cystine uptake could result in identification of tumors likely to be resistant to certain therapies; which could result in efficacious changes in treatment regimens.

An embodiment of the invention, comprises an imaging agent of Formula I



wherein X is ^{18}F , ^3H , $-(\text{CH}_2)_m\text{Z}$, $-\text{CH}_2-\text{O}-(\text{CH}_2)_m\text{Z}$, or $-\text{O}-(\text{CH}_2)_m\text{Z}$;

Z is ^{18}F or ^3H ;

m is an integer between 1 and 5;

Y is $\text{O}-\text{CH}_2$, CH_2-O , CH_2-CH_2 , $\text{CH}_2-\text{CH}_2-\text{CH}_2$, $\text{O}-\text{CH}_2-\text{CH}_2$, or $\text{CH}_2-\text{O}-\text{CH}_2$; and

Ar is aryl or heteroaryl.

Another embodiment of the invention comprises a method of imaging cells using the imaging agent Formula I. An example of the method generally comprises introducing into the target an imaging agent comprising ^{18}F or ^3H labeled

derivative of Formula I via the cystine/glutamate transporter; and detecting the imaging agent using one or more of, positron emission tomography (PET), autoradiography, scintillation detection, or a combination thereof.

Still another embodiment comprises a method of detecting oxidative stress in cells by introducing the ^{18}F or ^3H labeled derivative of Formula I via the cystine/glutamate transporter.

DETAILED DESCRIPTION

To more clearly and concisely describe and point out the subject matter of the claimed invention, the following definitions are provided for specific terms, which are used in the following description and the appended claims.

In the context of the present invention, alkyl is intended to include linear, branched, or cyclic hydrocarbon structures and combinations thereof, including lower alkyl and higher alkyl. Preferred alkyl groups are those of C_{20} or below. Lower alkyl refers to alkyl groups of from 1 to 6 carbon atoms, preferably from 1 to 4 carbon atoms, and includes methyl, ethyl, n-propyl, isopropyl, and n-, s- and t-butyl. Higher alkyl refers to alkyl groups having seven or more carbon atoms, preferably 7-20 carbon atoms, and includes n-, s- and t-heptyl, octyl, and dodecyl. Cycloalkyl is a subset of alkyl and includes cyclic hydrocarbon groups of from 3 to 8 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, and norbornyl. Alkenyl and alkynyl refer to alkyl groups wherein two or more hydrogen atoms are replaced by a double or triple bond, respectively.

Aryl and heteroaryl mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0-3 heteroatoms selected from nitrogen, oxygen or sulfur; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from nitrogen, oxygen or sulfur; or a tricyclic 13- or 14-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from nitrogen, oxygen or sulfur. The aromatic 6- to 14-membered carbocyclic rings include, for example, benzene, naphthalene, indane, tetralin, and fluorene; and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole and pyrazole.

Arylalkyl means an alkyl residue attached to an aryl ring. Examples are benzyl and phenethyl. Heteroarylalkyl means an alkyl residue attached to a heteroaryl ring. Examples include pyridinylmethyl and pyrimidinylethyl. Alkylaryl means an aryl residue having one or more alkyl groups attached thereto. Examples are tolyl and mesityl.

Alkoxy or alkoxyl refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration and combinations thereof attached to the parent structure through an oxygen. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, and cyclohexyloxy. Lower alkoxy refers to groups containing one to four carbons.

Acyl refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated and aromatic and combinations thereof, attached to the

parent structure through a carbonyl functionality. One or more carbons in the acyl residue may be replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, and benzyloxycarbonyl. Lower-acyl refers to groups containing one to four carbons.

Heterocycle means a cycloalkyl or aryl residue in which one or two of the carbon atoms are replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples of heterocycles that fall within the scope of the invention include pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzofuran, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, and tetrahydrofuran.

Substituted refers to residues, including, but not limited to, alkyl, alkylaryl, aryl, arylalkyl, and heteroaryl, wherein up to three H atoms of the residue are replaced with lower alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, haloalkyl, alkoxy, carbonyl, carboxy, carboxalkoxy, carboxamido, acyloxy, amidino, nitro, halo, hydroxy, $\text{OCH}(\text{COOH})_2$, cyano, primary amino, secondary amino, acylamino, alkylthio, sulfoxide, sulfone, phenyl, benzyl, phenoxy, benzyloxy, heteroaryl, or heteroaryloxy.

Haloalkyl refers to an alkyl residue, wherein one or more H atoms are replaced by halogen atoms; the term haloalkyl includes perhaloalkyl. Examples of haloalkyl groups that fall within the scope of the invention include CH_2F , CHF_2 , and CF_3 .

As used herein, the term "cystine/glutamate transporter" are used interchangeably with, and include, the terms cystine/glutamate antiporter, cystine/glutamate exchanger, cystine transporter, xc-, xc(-), Xc(-), system xc(-), and amino acid transport system Xc(-). The transport system comprises dimer of two proteins and includes, but is not limited to: protein xCT (SLC7A11) and protein CD98 (4F2hc, heavy chain of the 4F2 surface antigen, SLC3A2); protein xCT which is the subunit specific to the xc(-) system; protein CD98 which is a subunit common to a number of transporters with different substrates; and protein xCT that may also dimerize with rBAT, another subunit common to multiple transporters. Also the notations L-ASU and L-Asu both correspond to L-aminosuberic acid.

The cystine/glutamate transporter is not typically expressed or has extremely low expression in most tissues, but is upregulated in cells exposed to oxidative stress. Cystine, which comprises two disulfide-linked cysteine amino acids, is a natural substrate for this transporter. The effect of upregulation of the transporter is an increase in cystine uptake; which is then reduced to cysteine inside the cell. Intracellular cysteine is the rate limiting substrate for glutathione synthesis. Glutathione is the cells primary antioxidant to defend against oxidative stress. Intracellular cysteine is incorporated into one of two pathways, glutathione synthesis or protein synthesis.

As used herein, the term "radioisotopic label" includes, but is not limited to, radioisotopes that are used in a compound to trace or visualize the compound, or the mechanism of a chemical reaction, in a chemical or biological

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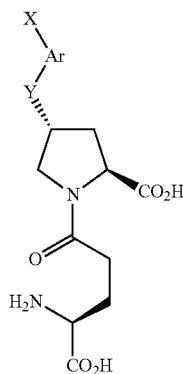
process, or biological substances, organisms and systems. Such labels are useful, for example, in connection with imaging and detection systems. Examples of suitable radioisotopic labels include, but are not limited to, ^3H , ^{123}I , ^{125}I , ^{131}I , ^{18}F , ^{11}C , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga and ^{68}Ga .

“Parenteral administration” refers to any means of introducing a substance or compound into a subject, that does not involve oral ingestion or direct introduction to the gastrointestinal tract, including but not limited to subcutaneous injection, intraperitoneal injection, intramuscular injection, intravenous injection, intrathecal injection, intracerebral injection, intracerebroventricular injection, intraspinal injection, intrathecal injection, intracerebral injection, intracerebroventricular injection, or intraspinal injection or any combination thereof.

“Pharmaceutical carrier” refers to a composition which allows the application of the agent material to the site of the application, surrounding tissues, or prepared tissue section to allow the agent to have an effective residence time for specific binding to the target or to provide a convenient manner of release. Solubilization strategies may include but are not limited to: pH adjustments, salt formation, formation of ionizable compounds, use of co-solvents, complexation, surfactants and micelles, emulsions and micro-emulsions. The pharmaceutical carrier may include, but is not limited to, a solubilizer, detergent, buffer solution, stabilizers, and preservatives. Examples of these include but are not limited to, HCl, citric acid, DMSO, propylene glycol, ethanol PEG 300, cyclodextrans, citrate, acetate, phosphate, carbonate or tris(hydroxymethyl)aminomethane.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Generally, the imaging agents of the invention comprise radioisotopic labeled analogs, that maintain the attributes necessary to be a substrate of the cystine/glutamate antiporter, represented by Formula I



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wherein X is ^{18}F , ^3H , $-(\text{CH}_2)_m\text{Z}$, $-\text{CH}_2-\text{O}-(\text{CH}_2)_m\text{Z}$, or $-\text{O}-(\text{CH}_2)_m\text{Z}$;

Z is ^{18}F or ^3H ;

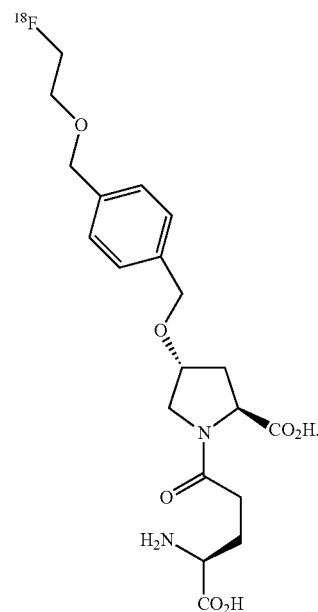
m is an integer between 1 and 5;

Y is $\text{O}-\text{CH}_2$, CH_2-O , CH_2-CH_2 , $\text{CH}_2-\text{CH}_2-\text{CH}_2$, $\text{O}-\text{CH}_2-\text{CH}_2$, or $\text{CH}_2-\text{O}-\text{CH}_2$; and

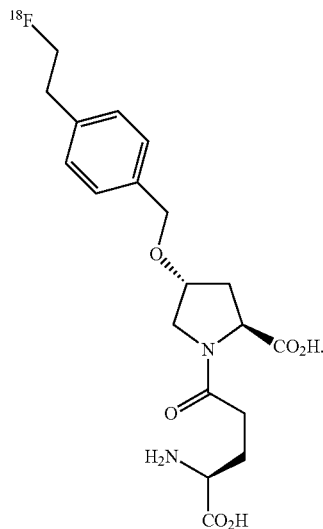
Ar is aryl or heteroaryl.

In certain embodiments X is $-(\text{CH}_2)_m\text{Z}$ whereby m is equal to 2 and Z is ^{18}F . In other embodiments, X is located at the para position relative to the Y moiety.

In one embodiment Formula I is:

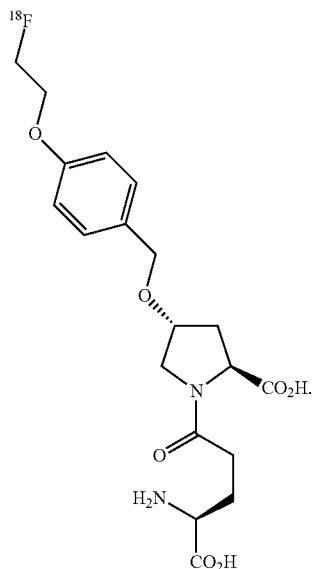


In another embodiment Formula I is:



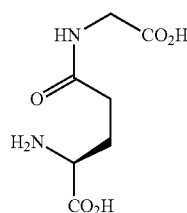
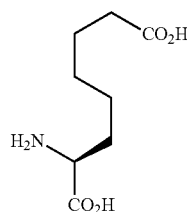
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In still another embodiment Formula I is:



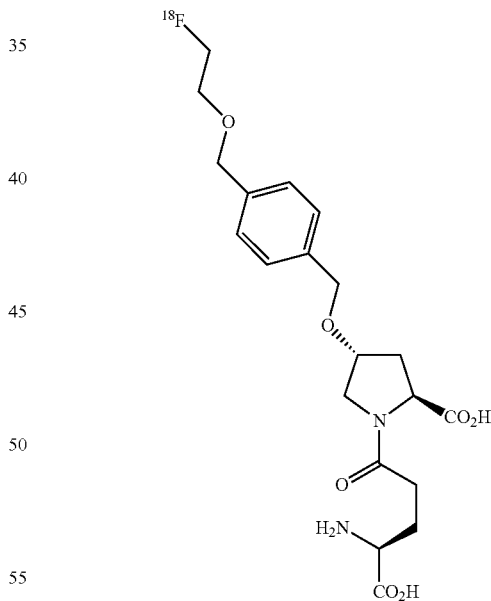
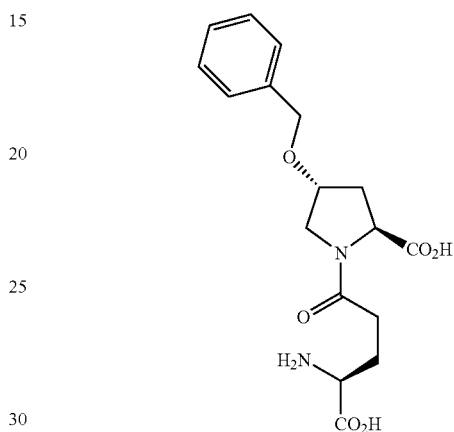
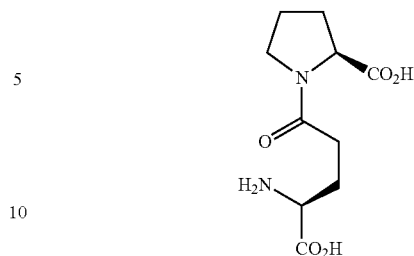
In designing the agent, as reported in aforementioned U.S. patent application Ser. No. 13/234,210 and shown in Scheme 1, aminosuberic acid (AUS,a) has good uptake via the oxidative stress transporter. It was found that putting an internal amide linkage in the chain (b) eliminates the entire uptake. However, making the amide from structure (b) more rigid by incorporating a ring such as in the proline analog (c) results in small but significant increase in uptake by the oxidative stress transporter. Uptake is then dramatically increased by substitution with an aryl group, either directly or through a flexible linker, at the 3 position of the proline (d). Structures of this type can be modified so that they are readily radiofluorinated under industry standard conditions, yielding PET agents such as structure e (Formula 1 $X=CH_2-O-(CH_2)_2^{18}F$).

Scheme 1; ASU backbone derivatives.



8

-continued



b

While, Formula I has 3 chiral centers, in certain embodiments, Formula I is limited to the L-isomer (levo-rotatory) stereochemistry at the glutamate stereocenter, comparable to the stereochemistry in the L-aminosuberic acid scaffold. The L-scaffold is distinctly different than other analogs in its class and has improved properties as a cystine/glutamate transporter imaging agent as shown in aforementioned U.S. application Ser. No. 13/234,210. Furthermore the stereochemistry of the hydroxyproline is 2S,4R.

In certain pharmaceutical application, the development of an individual enantiomer is preferred over racemates and racemic mixtures. Such mixtures usually require specialized chiral techniques for their correct identification, characterization, separation and measurement. They are often readily distinguished by biological systems, however, and may have different pharmacokinetic properties (absorption; distribution, biotransformation, and excretion) and quantitatively or qualitatively different pharmacologic or toxicologic effects. As such when stereoisomers are biologically distinguishable, the properties of the individual enantiomers should be characterized since racemates raises issues of acceptable manufacturing control of synthesis and impurities, adequate pharmacologic and toxicologic assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation. Thus the occurrence of Formula I as an individual stereoisomer is also advantageous, and the stereochemistry is controlled by the L-Glutamic acid and 2S,4R-hydroxyproline scaffolds.

The imaging agents may be detected by radioisotopic label. The method of detection of the compounds may include, but are not necessarily limited to, nuclear scintigraphy, positron emission tomography ("PET"), single photon emission computed tomography ("SPECT"), magnetic resonance imaging, magnetic resonance spectroscopy, computed tomography, or a combination thereof depending on the intended use and the imaging methodology available to the medical or research personnel.

The mechanism of cellular retention of these compounds will be by one of two mechanisms. For those compounds that are substrates for the transporter, when the labeled compounds are transported into the cell, they may not be incorporated into the protein synthesis or glutathione synthesis pathways. While it is possible that the compound could be transported out of the cell via the same transporter; the intracellular concentration of L-Glutamate is extremely high and therefore would be the favored substrate for cellular export, resulting in trapping of the majority of the compound in the cells. For those compounds that are not transporter substrates, the mechanism of cellular retention will relate to the affinity of the compound for the transport channel. These channel blockers will be retained as they physically block the transport channel and therefore will be limited to 1 to 1 stoichiometry of compound to transporter protein.

In a wide variety of human tissues and cells examined, the xc-transporter is predominantly expressed in brain, but also in pancreas and in cultured cell lines. The xc-transporter expression is very low in most tissues, but can be upregulated under conditions of oxidative stress and when cells are grown in culture. The xc-transporter is induced under a number of conditions, including apoptotic stimuli, oxidative stress, inflammation, cystine deprivation and chemotherapy resistance. For example, ^{18}F , may be used for in vivo PET imaging, as well as in vitro detection of cellular oxidative stress.

Similarly the upregulation of the cystine/glutamate transporter is also associated with chemotherapy resistance in some tumors. Therefore, non-invasive imaging of tumors with high cystine uptake could result in identification of

tumors likely to be resistant to certain therapies; which could result in efficacious changes in treatment regimens.

These agents that are taken up into cells may be used to image cellular oxidative stress in vivo, including without limitation, the imaging of pathologies or conditions that include cellular oxidative stress. Imaging applications that would benefit from these agents include, but are not limited to, chemotherapy treatment monitoring, ischemia/stroke, inflammation, traumatic brain injury and organ transplant monitoring.

The imaging agents of Formula I, can be incorporated into pharmaceutical compositions suitable for administration into a subject, which pharmaceutical compositions comprise a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible with the subject. The subject being a person or animal and the target being the cells or tissues of the subject, involved in the cystine/glutamate antiporter transport mechanism. Particularly, the carrier is suitable for intravenous, intramuscular, subcutaneous, or parenteral administration (e.g., by injection). Depending on the route of administration, the imaging agent of Formula I may be coated in a material to protect the compound or compounds from the action of acids and other natural conditions that may inactivate the compound or compounds and thus be introduced to the target.

In yet another embodiment, the pharmaceutical composition comprising the imaging agent of Formula I and a pharmaceutically acceptable carrier can be introduced to the target by being administered by combination therapy, i.e., combined with other agents. For example, the combination therapy can include a composition of the present invention with at least a therapeutic agent or drug, such as an anti-cancer or an antibiotic. Exemplary anti-cancer agents include cis-platin, adriamycin, and taxol. Exemplary antibiotics include isoniazid, rifamycin, and tetracycline.

In certain embodiments, the agent of Formula I is synthesized at a time sensitive or point of care location due to the decay of the material. As such, ease of manufacturing, purification, and handling are key in preparation a combination of a modular, automated, or micro-scale system is preferred. To accommodate such a need, in certain embodiments, the agent of Formula I is a single-label compound wherein a single radioisotope label is disposed as a substituent of the aryl moiety.

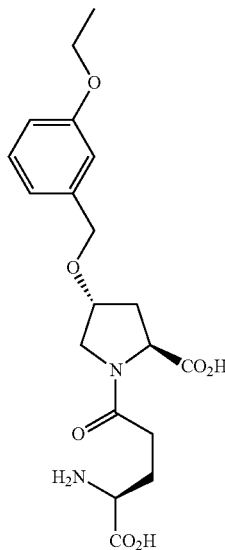
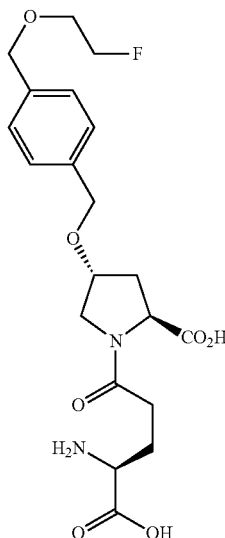
It is understood that the position of the radioisotope label may be positioned in other locations. In certain other embodiments, the compound may be labeled at multiple locations along the alkyl chain. However for ease of manufacturing in a time sensitive or point of care locations, the radioisotope label is a single moiety as shown.

Following are non-limiting examples used to illustrate various embodiments of the imaging agents and methods of use. As such Table 1 provides examples of the non-labelled analog of the final product. Also provided are measurements of inhibition response of ^3H -L-GLU (control) uptake in DEM treated EL4 cells. As such 96 well plate were seeded with 100,000 SKOV3 cells/well in complete growth media (McCoy's+10% Fetal Bovine Serum(FBS)) and incubated

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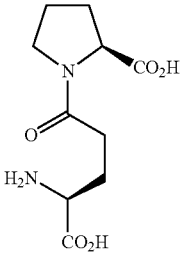
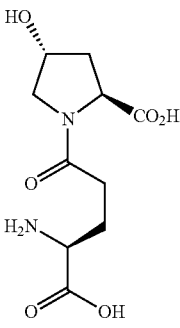
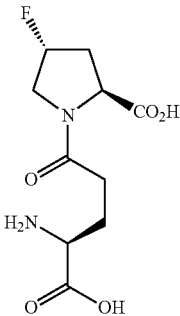
overnight at 37° C. The media was changed to 100 μ M DEM and again incubated overnight at 37° C. Wash 1 \times with Hank's Balanced Salt Solution (HBSS) Room Temperature and assayed 50 μ l of each agent (Table 1) in triplicate by incubating for 30 minutes (uptake) at room temperature. It was then washed twice with HBSS and 200 μ l MicroScint20 added for counting. The protocol used 200 uCi 3 H-Glu per well. The mix and agent and 3 H-Glu provided a final concentration of 200 uCi and 1 mM agent. The % of control, measured three times with standard deviation, shows uptake of 3 H-Glu. As such lower response is indicative of inhibition of the transporter. As such numbers below approximately 30% is equivalent to 70% inhibition and are considered good candidates for pharmaceutical agents.

TABLE 1

Non-radioactive agents and measurements of ^3H -Glu uptake			
Structure	% of control		Standard Deviation
	^3H -Glu uptake		
1q	14.49	0.72	
1r	10.06	1.59	

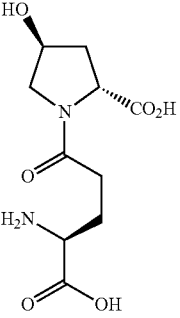
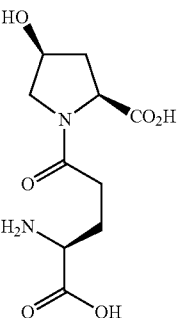
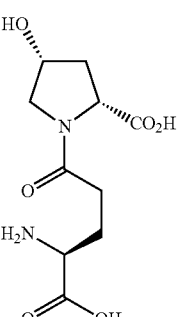
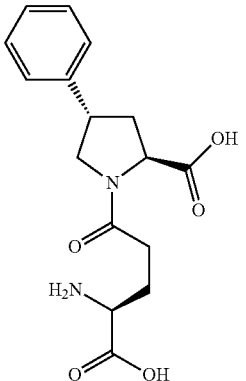
12

TABLE 1-continued

Non-radioactive agents and measurements of 3 H-Glu uptake			
Structure	% of control 3 H-Glu uptake	Standard Deviation	
1a	46.32	4.94	
1b	83.99	5.41	
1c	73.58	2.40	
1d	51.76	16.72	
1e			

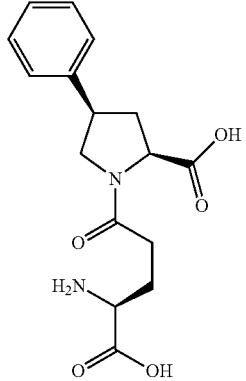
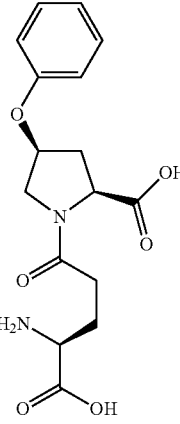
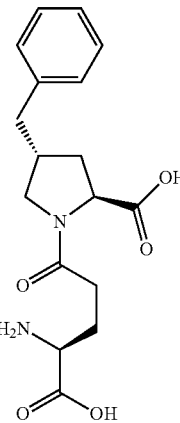
13

TABLE 1-continued

Non-radioactive agents and measurements of ^3H -Glu uptake			
Structure	% of control		Standard Deviation
	^3H -Glu uptake		
1e	74.05	8.33	
			
1f	30.81	3.05	
			
1g	94.69	1.36	
			
1h	52.29	1.43	
			

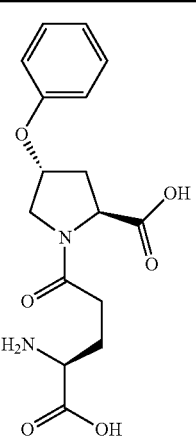
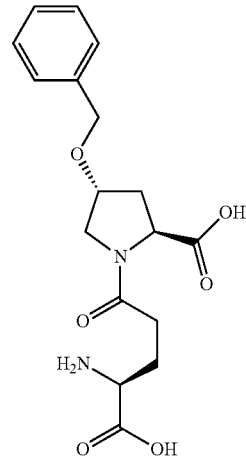
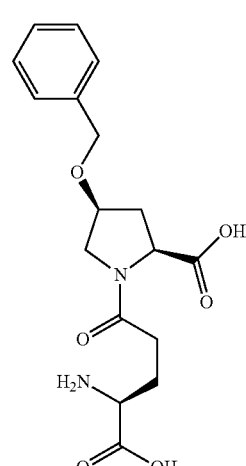
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TABLE 1-continued

Non-radioactive agents and measurements of ^3H -Glu uptake			
Structure	% of control		Standard Deviation
	^3H -Glu uptake		
1i	46.24	1.36	
			
1j	32.98	2.77	
			
1k	30.93	1.38	
			

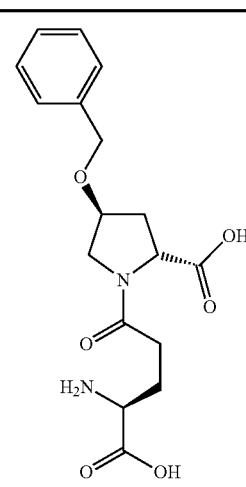
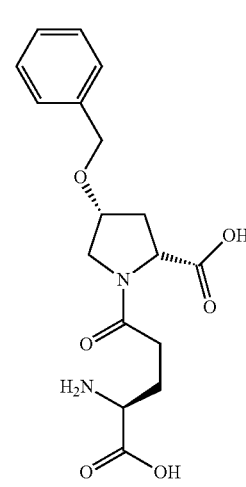
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TABLE 1-continued

Non-radioactive agents and measurements of ^3H -Glu uptake			
Structure	% of control ^3H -Glu uptake	Standard Deviation	
1l	36.64	5.12	
1m	14.89	2.90	
1n	26.16	2.00	

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TABLE 1-continued

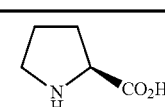
Non-radioactive agents and measurements of ^3H -Glu uptake			
Structure	% of control ^3H -Glu uptake	Standard Deviation	
1o	43.19	4.58	
1p	49.99	6.82	

As shown further in Table 2, the 2S,4R-hydroxyproline scaffold is preferred as such, structure 1m has improved uptake compared to structures 1n, 1o, or 1p. Furthermore the Aryl-methyl-ether is preferred over the Aryl-ether (structure 1m compared to structure 1l).

Experimental Procedures:

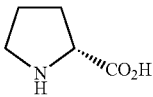
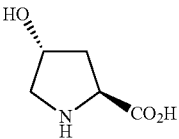
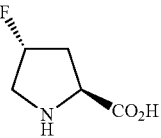
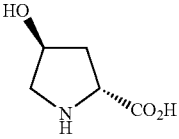
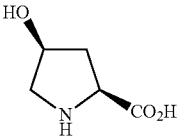
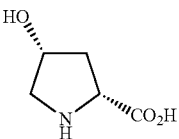
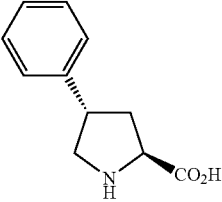
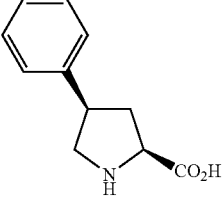
Table 2 represents the intermediate compound 3 in the synthesis below such that the structure and stereochemistry of the —R group remains.

TABLE 2

Intermediate 3	
a	

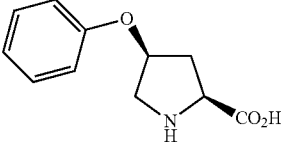
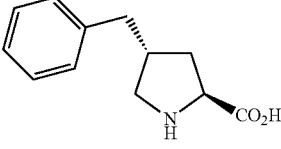
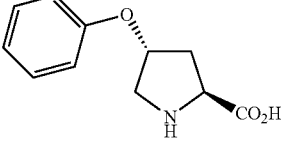
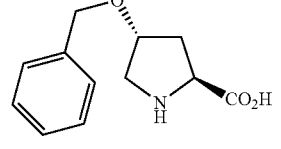
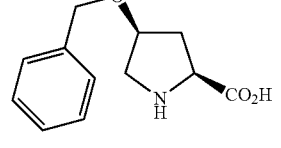
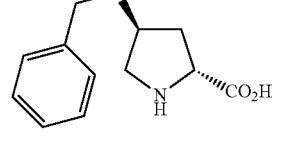
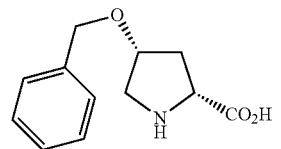
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TABLE 2-continued

Intermediate 3	
b	
c	
d	
e	
f	
g	
h	
i	

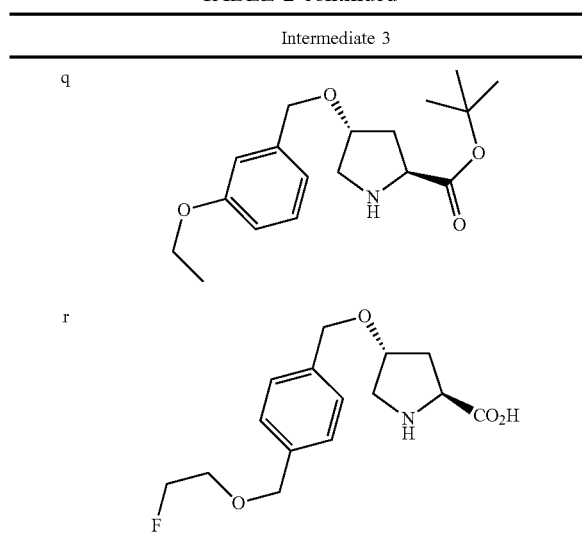
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TABLE 2-continued

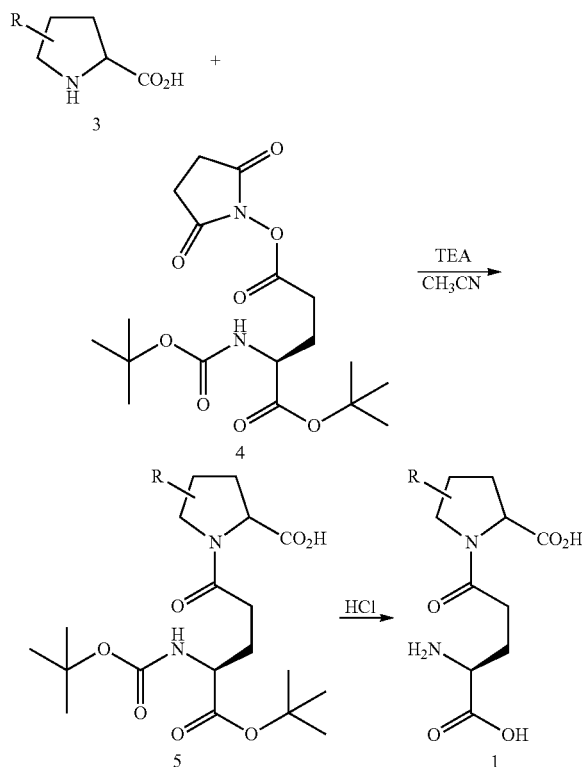
Intermediate 3	
5 j	
10 k	
15 l	
20 m	
25 n	
30 o	
35 p	
40	
45	
50	
55	
60	
65	

19

TABLE 2-continued



Synthesis of 1a-g

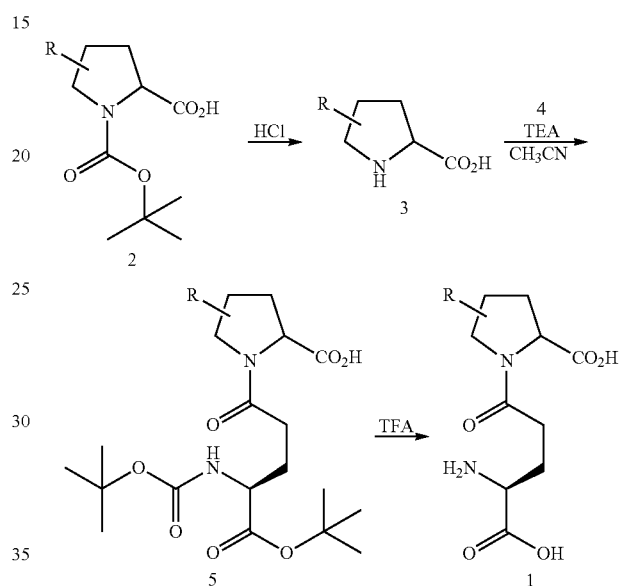


Dissolve or suspend 0.3 mmol of amine 3a-g in 100 μ L of water and 82 μ L of triethylamine in an 8 mL vial. For amine HCl salts add an additional 82 μ L of triethylamine. Add 100 μ L more water for any especially insoluble amines. Add 100 mg of Boc-Glu(OSu)-O-tBu (4, 0.25 mmol) in 1 mL of acetonitrile, cap the vial, and shake for 16 hours. Evaporate the acetonitrile under a nitrogen stream, add 1 mL of methylene chloride and enough 0.1 M aqueous HCl to bring the pH down to \sim 2.0 (\sim 4-5 mL). Separate the layers, and extract the aqueous layer twice with 1 mL portions of methylene chloride. Wash the combined organic layers with saturated

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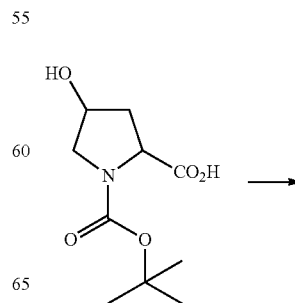
brine, strip off the solvent, and chromatograph each residue on a 4 gm silica column using a hexane/ethyl acetate gradient, yielding intermediate 5a-g, characterized by LCMS with positive ion mass spectrometry. The residues were then each dissolved in 1 mL of tetrahydrofuran, 1 mL of 4 M HCl in dioxane was added, and the each was stirred for 16 hours. The volatiles were then removed under reduced pressure, 2 mL of water were added to each, and each was lyophilized to yield the final product 1a-g as the HCl salt, characterized by LCMS with both positive and negative ion mass spectrometry.

Synthesis of 1h-1



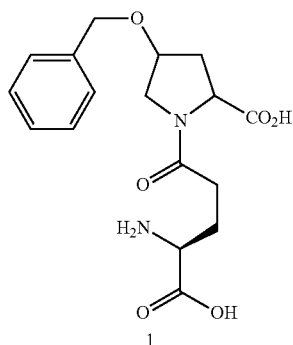
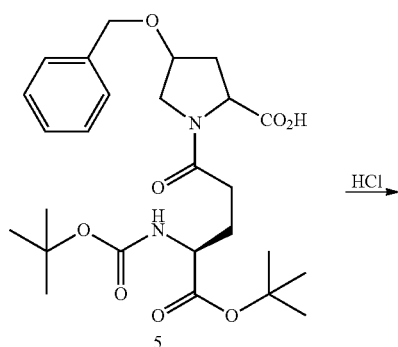
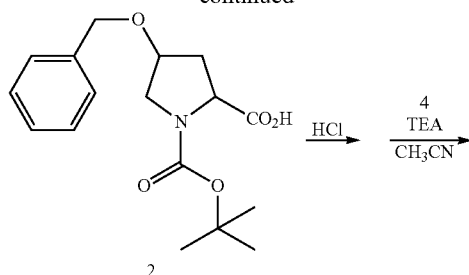
100-200 mg of Boc protected proline derivatives 2h-1 were dissolved in 2 mL of methylene chloride, 2 mL of 4M HCl in dioxane was added to each, and the mixtures were stirred for 16 hours. The volatiles were then removed under reduced pressure, and 0.3 mmol of each HCl salt was weighed out and converted to protected intermediate 5h-1 via the method described above for 5a-g. The final deprotection was done by dissolving the chromatographed residue in 1 mL of methylene chloride, adding 1 mL of trifluoroacetic acid, stirring for 16 hours, and removing the volatiles under reduced pressure. The residues were dissolved in 2 mL of water and lyophilized to yield the final products 1h-1 as TFA salts, characterized by LCMS with positive and negative ion mass spectrometry.

Synthesis of 1m-p



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-continued

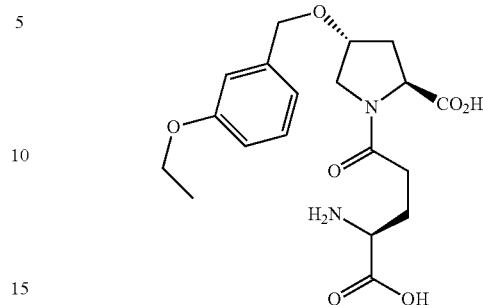


200 mg (0.86 mmol) of (2S,4R), (2S,4S), (2R,4R), or (2R,4S)-Boc-Hyp was dissolved in 3 ml of tetrahydrofuran, cooled to 0° C., and 103 mg (3 eq.) of 60% sodium hydride was added. The mixture was stirred cold for 20 minutes, then 226 μ l (2.2 eq) of benzyl bromide was added. The mixture was allowed to warm to room temperature and stirred for 16 hours, at which time it was cooled to 0° C. and 1 ml of water was added, followed by 500 μ l of 5% citric acid and 2 mL of saturated sodium bicarbonate solution. The organic layer was separated, and the aqueous phase was washed twice with 1 ml portions of ethyl acetate. The aqueous layer was then acidified to pH~2.0 with citric acid, and extracted three times with 2 ml portions of ethyl acetate. The combined organic layers were washed with saturated sodium chloride solution, the solvent removed under reduced pressure, the residues were dissolved in 2 ml of methylene chloride, and 2 ml of 4M HCl in dioxane was added. The mixture was stirred for 12 hours, and the solvent removed under reduced pressure, yielding the HCl salts 3m-p. 300 μ M of each was then converted first to 5m-p, then the final product 1m-p as the TFA salt via the methods described earlier for 1h-1.

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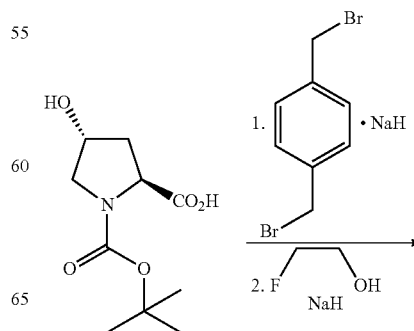
Synthesis of 1q

1q

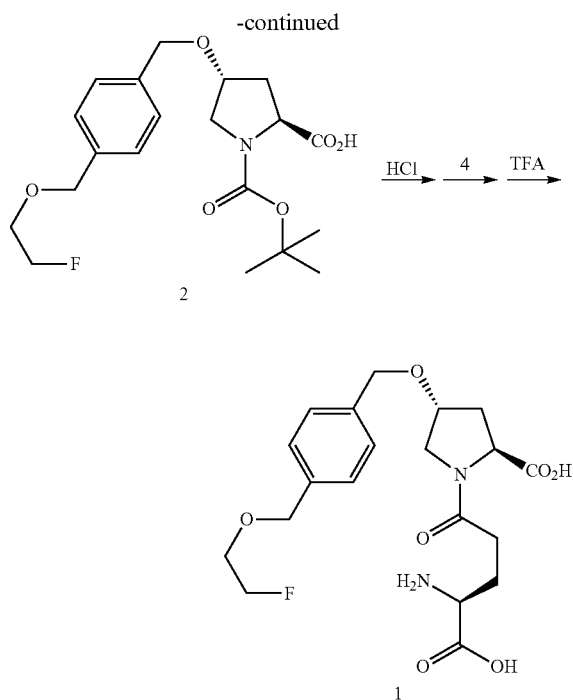


1 mmole of Boc-Hyp-OtBu was dissolved in 2 ml of DMF at 0° C. 1.1 mmole of 60% sodium hydride was added, the mixture was stirred for 20 minutes, then 1 mM of 1-(bromomethyl)-3-ethoxybenzene was added, and the reaction allowed to warm to room temperature while stirring for 16 hours. 5 ml of water was then added, and mixture was extracted 3 \times with 2 ml portions of 2:1 hexane:ethyl acetate. The combined organic layers were washed with saturated brine, the volatiles removed under reduced pressure, and the residue chromatographed on a 4 g silica gel column with a hexane/ethyl acetate gradient. The intermediate was then dissolved in 1 ml of ethyl acetate, 1 ml of 4M HCl in dioxane was added, and the mixture was stirred for 3 hours. The solvents were then removed under reduced pressure, 2 ml of saturated sodium bicarbonate solution was added to the residue, and the mixture was extracted with three 2 ml portions of methylene chloride. The solvent was removed from the combined organic layers, and the residue was chromatographed on a 4 g silica column using a hexane/ethyl acetate gradient, yielding 51 mg (0.158 mmol) of product. This was dissolved in 1 ml of acetonitrile, 50 μ l of triethylamine was added, then 53 mg of Boc-Glu(OSu)-OtBu. The mixture was stirred for 14 hours, the solvent was removed with a stream of nitrogen, and the residue partitioned between 1 ml of methylene chloride and 1 ml of water. The layers were separated, and the aqueous layer was extracted twice more with methylene chloride. The solvent was removed under reduced pressure from the combined organic layers, and the residue was chromatographed on a 4 g silica gel column with a hexane/ethyl acetate gradient. The product 5q was characterized by LCMS with positive ion mass spectrometry, and converted to the final product 1q as the TFA salt via the methods described earlier for 1h-1.

Synthesis of 1r:



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1 mmol of Boc-Hyp was dissolved in 40 ml of tetrahydrofuran at 0° C. 3 mmol of 60% sodium hydride was added, the mixture was stirred for 20 minutes, then 3 mmol of 1,4-bis(bromomethylbenzene) was added. The mixture was stirred for 16 hours while warming to room temperature. After cooling back down to 0° C., 6 mmol of 2-fluoroethanol was added followed by 6 mmol more of 60% sodium hydride. The mixture was stirred for 6 hours while warming to room temperature, then cooled back to 0° C. and quenched with 5 ml of water and 1 ml of 5% citric acid. 20 ml of saturated sodium bicarbonate solution was then added, and the layers were separated. The aqueous layer was washed twice with 10 ml portions of ethyl acetate, then acidified to pH<3.0 with solid citric acid. The mixture was extracted three times with 10 ml portions of ethyl acetate, the combined organic layers were washed with saturated brine solution, the solvent was removed under reduced pressure, and the residue chromatographed on a 4 g silica gel column with hexane/ethyl acetate as eluent, yielding 116 mg of product 2r. This was converted to the 1r TFA salt via the same method used for 1h-1, yielding 13 mg of material, characterized by LCMS with positive and negative ion mass spectrometry.

Synthesis of ^{18}F agent may be accomplished using standard practices for radiolabelling of materials. For example: a specific, non-limiting example of the method for synthesizing the monoAO- ^{18}F -FBA-Cystine is provided as follows.

All reactions were performed either under a nitrogen atmosphere or in a crimp-top sealed vial purged with nitrogen. Kryptofix 222 (Aldrich) and K_2CO_3 (EMD Science) were purchased and used as received. Optima™-grade acetonitrile was used as both HPLC and reaction solvents.

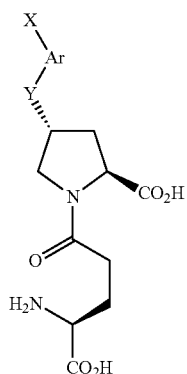
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^{18}F]KF (40 mCi/mL-1 (1480 MBq/mL-1) in purified water) was obtained from either IBA Molecular (Albany, N.Y.) or PETNET Solutions (Albany, N.Y.) and used as received. The ^{18}F]fluoride was first immobilized on a Chromafix 30-PS- HCO_3 anion exchange cartridge (ABX, Radeberg, Germany), then eluted into a drydown vessel with a 1 mL, 4:1 mixture of acetonitrile:distilled deionized water (ddH₂O) containing Kryptofix K222 (376 g·mol⁻¹, 8 mg, 2.13×10^{-5} mol) and potassium carbonate (138.2 g·mol⁻¹, 2.1 mg, 1.52×10^{-5} mol). The solvent was removed under partial vacuum and a flow of nitrogen with gentle heating (~45° C.) (~15 min). The source vial and anion exchange cartridge were then washed with 0.5 mL of acetonitrile containing K222 (8 mg) and the reaction mixture again brought to dryness under partial vacuum and gentle heating (~10 min). The reaction vessel was repressurized with nitrogen and the azeotropic drydown repeated twice with an additional 0.5 mL of acetonitrile. 4-formyl-N,N,N-trimethylanilinium triflate (313.30 g·mol⁻¹, 3.1 mg, 9.89×10^{-6} mol) was dissolved in 0.35 mL of anhydrous DMSO (Acros) and added directly to the reaction vessel containing the ^{18}F]KF.K222, K_2CO_3 . The reaction mixture was heated to 90° C. for 15 min and immediately cooled and quenched with 3 mL of distilled, deionized H₂O (ddH₂O). This mixture was subsequently passed through a cation exchange cartridge (Waters SepPak Light Accell Plus CM), diluted to 10 mL with ddH₂O, and loaded onto a reverse phase C18 SepPak (Waters SepPak Plus C18). The SepPak was flushed with 10 mL of ddH₂O then purged with 30 mL of air. ^{18}F]4-fluorobenzaldehyde (^{18}F]FBA), was eluted in 1.0 mL of methanol.

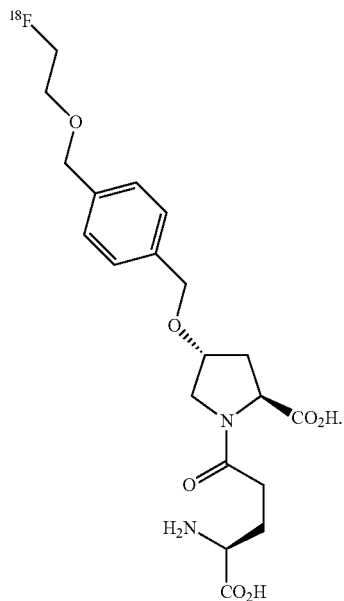
Separately, a high recovery vial (2 mL, National Scientific) was charged with mono-aminoxy cystine (386.27 g·mol⁻¹, 2.7 mg, 6.99×10^{-6} mol). The solid was suspended in 250 μL of ddH₂O and 8 μL of trifluoroacetic acid. 500 μL of ^{18}F]FBA in methanol (see above) was transferred to the reaction vial. The vessel was capped, crimped, placed in a heating block (activity at start of reaction 4.66 mCi/172 MBq) and maintained at 60° C. for 15 minutes; at which point a small aliquot (<5 μL) was removed for analytical HPLC analysis. 250 μL of ddH₂O with 0.1% TFA was used to dilute the solution to approx. 1000 μL , giving a final composition of 1:1 ddH₂O:MeOH in preparation for semi-preparative HPLC purification. ^{18}F]FB-Cystine was isolated and purified by semi-preparative HPLC. The HPLC fraction containing the product (0.409 mCi/15.1 MBq) was diluted 5:1 with ddH₂O and subsequently immobilized on a tC18 Plus Sep Pak (Waters). The SepPak was flushed first with 5 mL of ddH₂O then 30 mL of air. ^{18}F]FB-Cys (0.17 mCi, 6.3 MBq) was isolated in a minimal amount of DMSO by first eluting the void volume (approx. 0.5 mL) followed by collecting 250 to 300 μL of eluent in a separate flask. RP-HPLC analysis was performed on the isolated product in order to establish radiochemical and chemical purity. Typically, 10 μL of a 0.1 $\mu\text{Ci}/\mu\text{L}$ solution was injected for post formulation analysis. Isolated radiochemical yield was 3.6% (6.6% decay corrected from addition of ^{18}F]FBA) and radiochemical purity of 96.8%.

Synthesis of ^{18}H agent may be accomplished using standard practices for radiolabelling of materials. For example ^3H may be incorporated using a catalytic reduction with ^3H gas or introduced via reduction with isotopic hydride reagents or a transition metal-mediated exchange.

I

¹⁸F.

5. The agent of claim 1 wherein Formula I is



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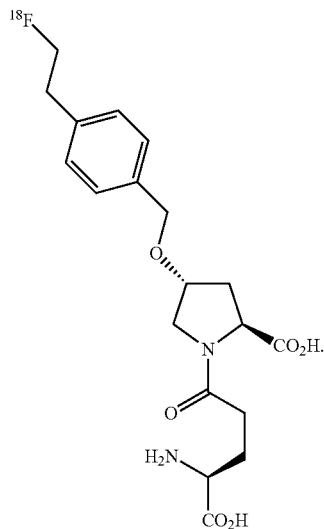
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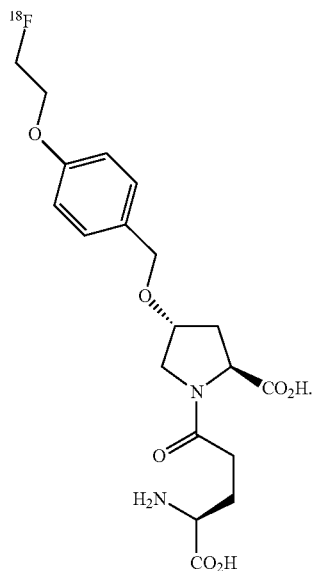
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6. The agent of claim 1 wherein Formula I is



7. The agent of claim 1 wherein Formula I is



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